A biochemical analysis of the fruit of *Tapinanthus leendertziae*

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In order to gain a clearer understanding of the mistletoe fruit/avian disperser interacting system the biochemical composition of the fruit of *Tapinanthus leendertziae* (Sprague) Wiens was studied. The information reported is the first of this type for any African mistletoe, and the first ever on the disperser-attracting component (the fleshy layer). The water, lipid, protein, fibre, ash, nitrogen-free extract and energy contents, as well as the fresh weight of the fleshy layer and the seed, including the viscin layer, were recorded. The protein and lipid (and thus the energy) contents are relatively high. Comparison with reports in the literature indicates a possible tendency to higher protein, lipid and energy contents in loranthoid species than in viscoid species. The exocarp accounts for 29% of the dry weight of *T. leendertziae* fruit, the fleshy layer for 13% and the seed, including the viscin layer, for 58%. Analysis of the fleshy layer showed that aspartic acid was the most abundant amino acid present (20%). The most important mistletoe disperser in the study area, the yellowfronted tinkerbird, *Pogoniulus chrysoconus* (Temminck), needs some 172 fruits of *T. leendertziae* per day to satisfy its energy requirements. The fruit belongs to the 'specialized' category of McKey (1975), ecological implications of which are discussed briefly.

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In 'n poging om meer duidelikheid te verkry oor die voëlentvrug/voëlverspreider interaksiesisteem, is die biochemiese samestelling van die vrug van *Tapinanthus leendertziae* (Sprague) Wiens ondersoek. Die inligting verskaf is die eerste van sodanige aard vir enige Afrikaanse voëlent, en die eerste ooit vir die verspreiderlokkingskomponent (die vlesige laag). Die water-, vet-, proteïen-, ruvesel-, as-, stikstofvrye-ekstrak- en energie-inhoud van die vlesige laag en die saad, met inbegrip van die viscinlaag, word gegee. Die proteïen- en vet- (en dus die energie-inhoud) is relatief hoog. Vergelyking met verslae in die literatuur dui op 'n moontlike neiging tot hoër proteïen-, vet- en energie-inhoude in loranthoïede spesies as in viscoïede spesies. Die eksokarp vorm 29% van die droëgewig van die vrug van *T. leendertziae*, die vlesige laag 13% en die saad, met inbegrip van die viscinlaag, 58%. Analise van die vlesige laag het getoon dat aspartiensuur die volopste aminosuur teenwoordig is (20%). Die belangrikste voëlentverspreider in die studiegebied, die geelkoptinker *Pogoniulus chrysoconus* (Temminck) benodig ongeveer 172 vrugte van *T. leendertziae* per dag om aan sy energiebehoeftes te voldoen. Die vrug behoort aan die 'gespesialiseerde' kategorie van McKey (1975). Die ekologiese implikasies hiervan word kortliks bespreek.

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Introduction

The relationships between mistletoes and their avian dispersers have not received much attention in southern Africa. This paper is the second one in a series describing botanical aspects of the mistletoe fruit/avian disperser interacting system (Godschalk 1983a,b); ornithological aspects are dealt with elsewhere (Godschalk in press b,c). For a clear understanding of these relationships, attention must among other things be given to the biochemical composition of mistletoe fruit as this is an essential factor in attracting the dispersal agents. Since fruit material was most readily available for *Tapinanthus leendertziae* (Sprague) Wiens its biochemical composition was determined. No biochemical analysis of South African mistletoe fruit has formerly been published.

Material and Methods

During March 1977 some 550 ripe fruits were collected from a *T. leendertziae* plant growing on an *Acacia caffra* (Thunb.) Willd tree in the Loskop Dam Nature Reserve, South Africa (19°19′E/25°26′S). After storage in deep-freeze for 15 months, the fruit were treated as follows. The exocarp was removed from each fruit and the remaining seed and fleshy layer were left together in one group (223 fruits). This sample was called 'fleshy-layer-seed'. In the other group (323 fruits) the fleshy layer was removed from the seed. The resulting two samples were called 'fleshy layer' and 'seed' respectively. All three sets of samples were analysed for total water, protein, lipid and fibre contents. Ash and energy contents were determined for 'seed' and 'fleshy-layer-seed' materials and the values for the 'fleshy layer' were computed from the 'seed' and 'fleshy-layer-seed' figures. The water content was also determined for the exocarps. The values are expressed on a dry-weight basis, unless otherwise stated.

Water content was determined by oven-drying to constant mass at 70 °C. Nitrogen content was determined by the macro-Kjeldahl method (Plummer 1971) and the resultant N value multiplied by 6,25 to get the protein content. Total lipid content was determined by Soxhlett petroleum ether extraction (Anon. 1965). The delipidized residue was used for the crude fibre determination and for an amino acid analysis of the fleshy layer. The crude fibre content was determined by digestion by acetic and nitric acids, and washing with ethanol and benzene. The residue was weighed

and this value was corrected by the ash content value to give the crude fibre content. The energy content was determined by means of a Phillipson microbomb calorimeter and the remaining residue was weighed and taken as the ash content. The nitrogen-free extract (NFE) was obtained by subtracting the percentage values of protein, lipid, crude fibre and ash from 100%. Amino acid analyses were carried out on a dry and on a delipidized sample of fleshy layers, by means of a Model 121M Beckman amino acid analyser.

Results

Data on the biochemical composition of the various components of *T. leendertziae* fruit are given in Table 1. The ash and energy values for the fleshy layer were derived indirectly from directly determined values for seeds and fleshy-layer-seeds. The differences between directly and indirectly obtained values for the fleshy layer in the case of protein, lipid and crude-fibre-and-ash determination (which could be checked), were only one, four and seven per cent, respectively, which suggests that the indirect derivations are reasonably reliable. The energy content could also be calculated indirectly by summing the energy contents of the different fractions using 23,64 kJ/g for protein, 39,54 kJ/g for lipids and 17,15 kJ/g for carbohydrates (including the NFE and crude fibre) as conversion factors (adapted from Paine 1971). This indirect energy estimation resulted in 25,49 and 26,73 kJ/g for the fleshy layer and the seed, respectively, which differs by 0,9 and 5,2%, respectively, from the values obtained by means of a microbomb calorimeter.

The red pigment in the fleshy layer is soluble in petroleum ether, being completely removed by Soxhlett extraction. The finely ground material of the seeds and fleshy-layer-seeds was extremely sticky and difficult to handle, because of the viscin. The viscin was apparently dissolved or neutralized, leaving behind a non-sticky powdery residue, during the petroleum-ether extraction process. This is unlike the findings of Schiller (1928) who found that material of *Loranthus europaeus* L. remained sticky after ether extraction.

The proportion of different amino acids in the protein fraction of the fleshy layer of *T. leendertziae* fruit is shown in Table 2. The results for the dry and the delipidized samples were nearly identical. Aspartic acid is the most important amino acid, constituting 20%.

Discussion and Conclusions

Only material of one plant growing on one host species was collected and analysed, which may make the results not completely representative. However, Tilney & Lubke (1974) found very few differences in chemical composition of material (stems and leaves) of *Viscum rotundifolium* L.f. collected from two different host species, *Boscia albitrunca* (Burch) Gilg & Benedict and *Ziziphus mucronata* Willd. This suggests that differences in composition of mistletoe fruit material (which is distal from the stems) collected from different host species should be minimal.

The data in Table 1 are apparently the first for any African species of mistletoe. They are apparently also the first for an analysis in which the attractant (the fleshy layer) for the disperser of mistletoe fruit has been treated separately. The protein content of the fleshy layer is higher than that of the seed, but the lipid content lower. The latter is probably important as a food supply for the embryo in the seed.

Table 1 Biochemical composition of various parts of the fruit of *Tapinanthus leendertziae*. Values are expressed as percentages per gram dry weight unless otherwise indicated and the figures in parentheses indicate the number of replicate determinations

	Seed $\bar{x}$ ± S. D.	Fleshy layer $\bar{x}$ ± S. D.	Exocarp $\bar{x}$ ± S. D.
Fresh weight (mg)	179,6 ± 8,00 (4)	78,7 ± 8,61 (4)	190,4 ± 13,26 (7)
% water (fresh)	51,5 ± 0,90 (3)	75,2 ± 1,20 (3)	77,1 ± 0,82 (7)
Energy (kJ/g)	25,40 ± 0,72 (5)	25,73[a]	
Protein	6,9 ± 0,22 (3)	9,1 ± 0,21 (2)	
Lipids	41,3 ± 1,06 (4)	34,7 ± 0,54 (2)	
NFE	50,2[a]	47,9[a]	
Crude fibre	0,9[a]	7,7[a]	
Ash	0,7 ± 0,84 (3)	0,6[a]	

[a]Value was derived indirectly (see text)

Table 2 A comparison of the amino acid composition of the protein fractions of the fleshy layer of fruit of *Tapinanthus leendertziae* and of whole fruit of *Loranthus europaeus* (data for the latter species obtained from Chiarlo & Cajelli 1965). Values expressed as percentages of total protein fractions, on a dry weight basis

Amino acids	*T. leendertziae* fleshy layer	*L. europaeus* fruit
Aspartic acid	19,6	16,5
Arginine	9,7	8,4
Glutamic acid	9,7	7,0
Proline	9,7	45,0
Leucine	7,6	1
Alanine	5,6	4,1
Serine	5,4	2,15
Valine	5,4	1
Lysine	4,8	9,2
Glycine	4,4	1,43
Threonine	4,4	1
Isoleucin	4,2	1
Phenylalanine	4,1	1
Histidine	2,8	–
Tyrosine	2,6	1,01
Cysteine	–	2,1
Methionine	–	1

Tomann (1906) and Schiller (1928) found an ample amount of fat drops in the viscin of *Loranthus europaeus*. If a similar situation is found in *T. leendertziae* this may be a contributing factor to the higher lipid content of the seed (which includes the viscin layer). The reason for the high fibre content of the fleshy layer is not clear. During March 1977 I observed a plumcoloured starling, *Cinnyricinclus leucogaster*

(Boddaert), regurgitating two pellets consisting of fibrous remains of fleshy layers of *T. leendertziae* fruit (Godschalk 1979). This indicates the removal of excessive fibrous material. This behaviour was never observed in any other mistletoe-fruit-eating bird species in the study area. The energy values of the fleshy layer and the seed are nearly identical. Included in the seed is, of course, also the viscin layer which seems to consist mainly of pectose in *L. europaeus* and of pectose and cellulose in *V. album* L. (Gjokic 1896; Tomann 1906; Schiller 1928; Mangenot *et al.* 1948). In the case of *T. leendertziae* 29% of the dry weight of the fruit (42% of the fresh weight) is invested in the exocarp for protection during maturation; 13% (18%) in the fleshy layer for attraction of dispersal agents and 58% (40%) in the seed, partly for attachment and partly for the embryo of the next generation. In comparison, in *V. combreticola* Engl. the exocarp accounted for 66% of the fresh weight of the fruit, the seed and fleshy layer together contributing only 34% ($n = 100$; 13,2 g; Godschalk 1979). As the main function of the exocarp is the protection of the fruit against predispersal predation, it can be speculated that the fruit of *V. combreticola* is subjected to more such predation than *T. leendertziae* and especially the *Viscum* species with thin exocarps (Godschalk 1983a) but no data are available on this aspect.

From earlier reports in the literature, it is not always clear which fractions are comparable to those obtained in more recent analyses, because the methods of analysis were not standard. Hence, care is needed in comparing results. Schiller (1928) found that lipids comprise 36% (dry weight) of whole *L. europaeus* fruit, which is similar to my results with *T. leendertziae*. Walsberg (1975) found 15% lipids and 7,5% protein, on a dry-weight basis, in whole fruit of *Phoradendron californicum* Nutt. (a viscoid species). The energy content was 22,09 kJ/g dry weight. Crome (in appendices to his 1975 paper) reports 8,29% protein and 4,5% lipids, on a dry-weight basis, in the 'flesh' (i.e., seed and fleshy layer) of fruit of *Notothixos subaureus* Oliv. (a viscoid species), which made up 96,7% of the whole fruit, a condition similar to that found in some South African *Viscum* species with thin exocarps (Godschalk 1983a). The reported protein values for *P. californicum* and *N. subaureus* are similar to those in *T. leendertziae*, but the lipid contents of the former two species are considerably lower. The energy content of *P. californicum* fruit was much lower than in the fleshy layer or the seed of *T. leendertziae*, which can be attributed to the relatively low lipid content of the former species. Because no previous analyses of mistletoe fleshy layers have been carried out, it is difficult to make general comparisons. The much lower reported lipid values for two viscoid species and the higher values in two loranthoid species may, however, indicate differences in the nature of the fruit between the two taxa. The fleshy layers of *V. combreticola* and other *Viscum* species are composed of a jelly-like substance (Godschalk 1983a) and presumably have a lower lipid content than those of loranthoids. The reported presence of a fairly large amount of fat drops in the viscin of *L. europaeus* in contrast to the virtual absence thereof in *V. album* (Tomann 1906; Schiller 1928) further substantiates the trends of higher lipid contents in loranthoid fruit in comparison with those of viscoid species. Differences in fruit morphology of Loranthaceae and Viscaceae are reported elsewhere (Godschalk 1983a), while Tilney & Lubke (1974) reported differences in their phenolics.

To the author's knowledge, the only other detailed analysis of amino acids of mistletoe fruit was carried out by Chiarlo & Cajelli (1965) on whole fruit of *Loranthus europaeus*. Again, care should be taken in making comparisons, as different types of material (fleshy layers and whole fruit, respectively) were analysed, but it appears that *T. leendertziae* fleshy layers have low proline and high leucine and valine contents in comparison with *L. europaeus* fruit (Table 2).

Ecological implications

Using King's (1974) tentative equation for total daily energy expenditure (DEE) in free-living birds, the number of fruits of *T. leendertziae* needed for providing the energy requirements of the yellowfronted tinkerbird, *Pogoniulus chrysoconus* (Temminck) (the main disperser of mistletoe seed in the study area, Godschalk in press b), can be estimated roughly. King's equation reads: $DEE = 317{,}7 + W^{0{,}7052}$, where DEE is in kcal/day and W is body weight in kilograms. With W specified as 0,0125 kg, the estimation of DEE is 14,45 kcal (60,208 kJ) per bird per day, which means that 120,4 fruits of *T. leendertziae* are needed to satisfy the bird's energy requirements, at 100% efficiency. It seems reasonable to assume a digestive efficiency of 70% since Walsberg (1975) found 49% energy utilization efficiency in *Phainopepla nitens* (Swainson) feeding on mistletoe fruit. However, he included the whole fruit, while the seed is not digested at all and in this case 172 fruits per bird per day would be needed. In the present calculation it was assumed that the bird takes only mistletoe fruit for its energy requirements, whereas, in fact, insects are also taken (Godschalk 1979). The actual number of fruits consumed may thus be lower. Unfortunately, however, no data on the actual intake of fruits per day are available. The fleshy layers of 172 fruits of *T. leendertziae* contain 10,2 g water (81% of the bird's body weight), which is more than enough for the bird's daily water requirements.

McKey (1975) recognized different basic seed dispersal strategies in plants whose seeds are dispersed by fruit-eating birds. This concept was extended by Howe and Estabrook (1977) who recognized two main patterns of seed dispersal by birds: specialized and generalized. McKey (1975) stated that mistletoes, in general, belong to the group of so-called 'specialized' fruits dispersed by 'specialized' frugivores (Howe & Estabrook 1977). These specialized fruits characteristically have highly nutritious arils or fleshy layers. Snow (1971) reports mean lipid and protein contents, on a dry-weight basis, for six British 'generalized' fruits as 5,4% and 9,2%, respectively, in contrast to 29,7% and 11,6%, respectively, for five tropical fruits of the specialized type. Five succulent fruits had only traces of lipids and 4,2% protein contents (Snow 1962). Morton (1953) reports a mean protein content of 54,0% for five species of insects, and 10,4% lipids. The aril of *Stemmadenia donnell-smithii* (Rose) Wendson (a specialized Costa Rican fruit) contains 63,9% lipids and 10,95% protein (McDiarmid *et al.* 1977).

Yellowfronted tinkerbirds thus apparently feed on mistletoe fruit for the high lipid (energy) content and presumably add insects to their diet for supplementing their protein requirements. Comparison of the above-mentioned data with those obtained for *T. leendertziae* immediately shows that the latter belongs to the category of specialized fruit. This fact is discussed in broader context elsewhere (Godschalk in press a).

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References

ANONYMOUS. 1965. Official and tentative methods of analysis of the association of official agricultural chemists, 10th edition.

CHIARLO, B. & CAJELLI, E. 1965. Fatty acids and amino acids in the berries of *Loranthus europaeus*. *Boll. Chim. Farm.* 104(11): 735 – 743. (Italian; data obtained from *Chem. Abstr.* 64: 8638 (1966).)

CROME, F.H. 1975. The ecology of fruit pigeons in tropical Northern Queensland. *Aust. Wildl. Res.* 2: 155 – 185.

GJOKIC, G. 1896. Zur Anatomie der Früchte und des Samens von *Viscum*. *Sber. Akad. Wiss. Wien*, Math.-naturw. kl., Abt.I; 105: 447 – 464.

GODSCHALK, S.K.B. 1979. The dispersal of three mistletoe species by birds in the Loskop Dam Nature Reserve. Unpublished M.Sc. thesis, University of Cape Town.

GODSCHALK, S.K.B. 1983a. The morphology of some South African mistletoe fruits. *S. Afr. J. Bot.* 2: 52 – 56.

GODSCHALK, S.K.B. 1983b. The reproductive phenology of three mistletoe species in the Loskop Dam Nature Reserve, South Africa. *S. Afr. J. Bot.* 2: 9 – 14.

GODSCHALK, S.K.B. in press a. Mistletoe dispersal by birds in South Africa. In: The Biology of Mistletoes, eds Calder, D.M. & Bernhardt, P. Ch. 7. (1983).

GODSCHALK, S.K.B. in press b. Birds eating mistletoe fruit in the Loskop Dam Nature Reserve, South Africa. *S. Afr. J. Zool.*

GODSCHALK, S.K.B. in press c. Feeding behaviour of mistletoe-eating birds in the Loskop Dam Nature Reserve, South Africa. *S. Afr. J. Zool.*

HOWE, H.F. & ESTABROOK, G.F. 1977. On intra-specific competition for avian dispersers in tropical trees. *Am. Nat.* 111: 817 – 832.

KING, J.R. 1974. Seasonal allocation of time and energy resources in birds: 4 – 85. In: Avian energetics, ed. Paynter, R.A. *Publs Nuttall orn. Club* 15, Cambridge, Massachusetts.

McDIARMID, R.W., RICKLEFS, R.E. & FOSTER, M.S. 1977. Dispersal of *Stemmadenia donell-smithii* (Apocynaceae) by birds. *Biotropica* 9: 9 – 25.

McKEY, D. 1975. The ecology of coevolved seed dispersal systems: 159 – 191. In: Coevolution of animals and plants, eds Gilbert, L.E. & Raven, P.H. University of Texas Press, Austin and London.

MANGENOT, G., REBIFFE, J. & ROUDIER, A. 1948. Sur le mucilage du gui. *C. r. Acad. Sci., Paris*, 227: 439 – 441.

MORTON, E.S. 1973. On the evolutionary advantages and disadvantages of fruit eating in tropical birds. *Am. Nat.* 107: 8 – 22.

PAINE, R.T. 1971. The measurement and application of the calorie to ecological problems. *Annu. Rev. Ecol. Syst.* 2: 145 – 164.

PLUMMER, D.T. 1971. An introduction to practical biochemistry. McGraw-Hill, London.

SCHILLER, F. 1928. Zur Kenntnis der Frucht von *Viscum album* und *Loranthus europaeus* und der Gewinnung von Vogelleim. *Sber. Akad. Wiss. Wien*, Math.-naturw. kl., Abt.I; 137: 243 – 258.

SNOW, D.W. 1962. The natural history of the oilbird, *Steatornis caripensis* in Trinidad. Part 2. Population, Breeding Ecology and Food. *Zoologica* 47: 199 – 221.

SNOW, D.W. 1971. Evolutionary aspects of fruit eating by birds. *Ibis* 113: 194 – 202.

TILNEY, P.M. & LUBKE, R.A. 1974. A chemotaxonomic study of twelve species of the family Loranthaceae. *Jl S. Afr. Bot.* 40: 315 – 332.

TOMANN, G. 1906. Vergleichende Untersuchungen über die Beschaffenheit des Fruchtschleimes von *Viscum album* L. und *Loranthus europaeus* L. und dessen biologische Bedeutung. *Sber. Akad. Wiss. Wien*, Math.-naturw. kl., Abt.I; 115: 353 – 365.

WALSBERG, G. 1975. Digestive adaptations of *Phainopepla nitens* associated with the eating of mistletoe berries. *Condor* 77: 169 – 174.